BNJAR

Blue Nile Journal of Agricultural Research (BNJAR)
Vol. 1, Issue. 1, June, 2020, pp. 13-23

Journal homepage: https://www.arari.gov.et/index_bnjar.php

Resin Secretory Structures of Boswellia Neglecta (Burseraceae) From The Borana Zone in Southern Ethiopia

Getabalew T. Reta[1*] and Motuma F. Tolera[2]
[1]Debere Birhan Agricultural research center, PO Box 112, Debere Birhan, Ethiopia
[2]Hawassa University, Wondo Genet College of Forestry and Natural Resources, P.O. Box 128, Shashemene, Ethiopia
[*]Corresponding Author email: getabalew2007@gmail.com,

Received: February 17, 2020
Revised: May 30, 2020
Accepted: June 25, 2020
Available online: June 29, 2020

Keywords: Boswellia neglecta, frankincense, resin-secretory structures, axial resin canals.

ABSTRACT

Boswellia neglecta is a small tree that produces commercially important oleo-resin known as frankincense. This frankincense has been used for varied purposes, and it is a source of income for rural households in southern and south-eastern Ethiopia. Despite the long history of using this species, knowledge of bark anatomy and resin secretory structures is absent. In this study, anatomical description of the bark including network and distribution of resin secretory structures were determined; relationship between bark thickness, and axial resin canals characteristics with tree DBH of B. neglecta were assessed in dry deciduous woodlands of Ethiopia, Borana zone. Twenty-one trees were selected systematically for this study. The network of resin-secretory structure was investigated from tangential, radial and transversal sections of bark samples using light microscopy. The average density, diameter and total area of axial resin canals were determined from transversal sections of 42 bark samples. The results of the study showed that in the inner bark axial and radial resin canals occurred and that they are interconnected by radial resin canals. Whereas, in the wood, there was not any resin secretory structures. On average, density of functional-axial resin canals is low, reaching 0.33 mm^{-2}. Inner bark thickness linearly increases with tree diameter, and resin canal density was not varying across the inner bark. Total area of axial resin canals was highly significant ($p<0.001$) positively related to the DBH. These results will lead to new advances in our knowledge of the resin secretory structures of B. neglecta.

1. INTRODUCTION

Several tree species are known to produce resins in tropical and subtropical zones (Langenheim, 1994). Commercially valuable resins are produced from few families, (e.g., *Burseraceae*, *Pinaceae* and *Leguminosae*) (FAO, 1995). Resins from *Boswelli*a and *Commiphora* species are recognized as frankincense and myrrh, respectively. The resins are used for different ceremonies (Groom, 1981; Tucker 1986a), raw material for several industries (Lemenih and Teketay, 2003), pharmaceuticals (Greene, 1993), food industries (Ford et al., 1992; Khan and Abourashed., 2011), and cosmetic industries (Khan and Abourashed, 2011).

Frankincense is produced and exported by few countries. Somalia and Ethiopia are the major producers and exporters in the world markets (Wahab et al., 1987). In Ethiopia, resin can be obtained from several tree species. Three types of Frankincense products are recognized in Ethiopia, namely Tigray, Ogaden and Borena. The Tigray type is the resin obtained from B. papyrifera. This frankincense produced in the west, north and north-western parts of the country (Lemenih and Kassa, 2011). The Ogaden types represent frankincense produced in the eastern parts of the Ethiopia. The Borana-type frankincense is produced from *Boswellia neglecta* growing in the southern and south-eastern part of the country (Eshete et al., 2005; Tadesse et al., 2007a). This frankincense has been used for various purposes, and it is a source of income for rural households (Eshete et al., 2005; Worku et al., 2011). It is widely traded as incense on local markets (Worku, 2006).

The southern part of Ethiopia, predominantly the Borana zone, is endowed with gum and resin producing species (Gemedo-Dalle et al., 2005; Worku et al., 2012). Among these, *Boswellia neglecta* is ecologically adapted, commercially and socio-culturally preferred species. The species has multiple uses such as charcoal, fuel wood, construction wood, medicine, and oleo-gum resin for frankincense (Eshete et al., 2005; Gizaw, 2006). Frankincense is important to generate income at household thereby to improve the livelihoods (Worku, 2006).

The resins are characterized by the nature of the species, method of tapping and stae to wounds/injuries. However, the structures of the plant part vary from species to species (Langenheim, 2003; Nagy et al., 2000). Tolera et al. (2013a) investigated the wood and bark structure of *Boswellia papyrifera* in the North-western Ethiopia. They found that both axial and radial resin canals are abundant near the cambium, but decrease in number when increasing the distance from the cambium to outwards. (Bhatt, 1987) found that both axial and radial canals are found in the bark of *Commiphora wightii (Burseraceae*). In addition, tree size characteristics (DBH, leaf apices and age) affected the characteristics of the resin (Tolera et al., 2013b). These features are also closely related to resin yield. This result is supported by (Blanche et al., 1992).

So far, no comparable research has been done on *B. neglecta*. The limitation of information is an obstacle to develop sustainable tapping technology. Currently, there is an increasing need to introduce tapping technology. The non-timber forest product case team at Forestry Research Centre (FRC) would like to conduct a series of study to develop tapping technologies for various gum and resin producing species in Ethiopia. Understanding the type, distribution and networking of resin secretory structures is important to develop tapping technologies (Bannan, 1936; Tolera et al., 2013). Hence, this study is initiated as part of the an effort by Forestry Research Centre (FRC) and it aims to describe the bark and resin secretory structures in the bark and wood of B. neglecta, an species from which Borana type frankincense is predominantly produced.

B. neglecta naturally oozed white or black resin from their bark during the dry seasons (Personal observation). This leads the presence of resin-secretory structures (axial and radial resin canals) may appear in the bark and probably also the wood of *B. neglecta* species. It is hypothesized that the distribution and density of axial resin canals exhibit directional changes in the inner bark as a result of dilatation similar to that of *B. papyrifera* (Tolera et al., 2013). The objectives of this study were to investigate the bark structure and resin canals distributed in *B. neglecta* and to examine the relationships between bark thickness and resin secretory structure.

2. Materials and Methods

2.1. Description of the study area

The study area is located in the Borana zone of Oromia regional state, southern Ethiopia. The research is conducted in Arero district, situated south east of Borana at distance of 660 km south of Addis Ababa (Angassa and Oba, 2008). The average annual rain-fall ranges 400 - 600mm (Gemedo-Dalle et al., 2005) The rainfall is bimodal distribution with a main dry season that occurs between December and February, and a long rainfall falling in April and May (Gemedo-Dalle et al., 2005). The dominant soil types in the district are granitic and volcanic with *Acacia-commiphora* woodlands dominated by *Acacia*, *Commiphora* and *Boswellia* species (Worku et al., 2012). *Acacia*, *Commiphora* and *Boswellia* species are the main source of oleo-gum resins (Eshete et al., 2005)

2.2. Sampling techniques

Sample trees were selected from 12 quadrants of 50x50m lied along three transect lines in woodland dominated by *B. neglecta*. The quadrants were from a square grid of 500m between the quadrants and same distance (500m) between the transect lines. The direction of transect lines and the distance to first quadrant was selected randomly. Stem diameter of all individual trees of *B. neglecta* were measured and coded at breast height (DBH) i.e., at 1.3 m above ground). After measurement, trees classes were formed using DBH (1=5-7cm, 2=7.1-9 cm, 3= 9.1-11, 4=11.1-13 and 5= 13.1-15, 6=15.1-17, 7= ≥ 17.1). Then, three trees per diameter class were sampled randomly. Two bark samples per tree were taken from the opposite side at breast height with a Trephor tool (a single, compact and easily transported tool; size: 140 mm long and 5 mm in diameter) from a sampled of 21 *B. neglecta* trees. For this study, 42 bark samples were collected. Investigation of resin-secretory structures in wood of *B. neglecta* was done on samples from stem discs of seven trees. To avoid fungal deterioration, the collected samples were kept in plastic tubes with a 70% ethanol solution.

2.3. Micro-thin section preparation

In the Wondo Genet College of Forestry and Natural resource, wood science laboratory, micro-thin sections of 50-µm thickness were prepared from transversal sections of all 42 bark samples (three bark samples per diameter class), and tangential and radial thin sections were prepared from seven bark samples (one bark sample per diameter class randomly selected) by using A sliding microtome (type: G. S. L.I light- weight microtome, sliding range: 300mm, specimen thickness: 2 to 30 micron; object clamp: 38x48mm, Dimensions: 37x24x20 cm). And also, micro-thin sections (50 µm thickness) were prepared from transversal, tangential and radial sections of seven stem discs to check the presence or absence of resin secretory structures in the wood of *B. neglecta*. The thin sections were stained with a mixture of Astra blue and safranin (150mg Astra blue, 40mg safranin and 2 ml acetic acid in 100 ml distilled water) for 15 minutes for microscopic observations and to distinguish functional (living) tissues from non-functional (dead) tissues Schweingruber et al. (2006). Next, the stained micro-thin sections were cleared by distilled water, and subsequently the water droplets were dehydrated by glycerin solution. In general, this procedure was helping to prepare temporary slides. The prepared slides were transported to Wageningen University, wood science laboratory in the Nether Lands for further examination of resin-secretory structures.

2.4. Examination of resin-secretory structures

The type, network, distribution and characteristics of resin-secretory structures in the bark as well as presence/absence of resin-secretory structures in the wood were investigated from transversal, tangential and radial sections of the micro- thin sections of bark and wood samples using a light microscope (Leica DM 2500, type HC PL Fluotar Ph1; for midum a numerical apertur of 0.30) with magnification ranges from 1.25 to 40x. Digital images of resin-secretory structures such as, axial and radial resin canals were taken from 42 transversal, 7tangential and 7radial micro-thin sections using a light microscope attached with Leica camera(size 138x77x38, M6, magnification0.85) (Tolera et al.,2013).

The resin canals were classified as functional and non-functional resin canals according to the techniques illustrated by Tolera et al. (2013). Functional and non-functional resin canals were distinguished according to the presence or absence of lignification of the cell wall of epithelial cells by using light microscopy. If a resin canals was encircled by non-lignified cell walls of epithelial cells, it was considered as functional resin canal; if the resin canals encircled by lignified cell wall of epithelial cells, it considered as non-functional resin canals.

2.5. Determination of bark cross-sectional area

The width and thickness of the inner bark of each bark sample as well as total bark thickness (inner + outer bark) were measured and averaged for the two bark samples per tree following the method applied by Tolera et al. (2013) using Image J software (version 1.44p). The inner bark- area of individual bark samples was determined from the width and thickness (inner bark area =tangential width of the bark sample * thickness of the bark). The bark cross-sectional area at the sampling height (DBH) of each sample tree was estimated from the total cross-sectional area of the sample tree and the average bark thickness as calculated from the two samples.

This mean that bark cross-sectional area = total cross-sectional area - its cross-section- area of wood (assume circular shape at point of sampling). Where by cross-sectional area of wood= $((DBH/2) - \text{average bark thickness})^2 * \pi$.

2.6. Measurement of resin-canal characteristics

Resin-canal characteristics were measured from transversal thin sections made from the two bark samples per tree. All axial resin canals were counted and their diameter was measured across the inner bark by using Image analysis software (Image J version 1.44p).

Average resin-canal density, diameter and total area of axial resin canals were measured from 21 study tree samples (2 bark samples per tree) of *B. neglecta*, following the methods showed by Tolera (2013b*)*. The average density of axial resin canals per inner-bark area (mm^{-2}) was calculated by dividing the total number of axial resin canals by the measured inner bark area of the sample. For each tree, an average resin-canal density is calculated from the measurements collected from the two samples. Likewise, the average diameter of the axial resin canals was determined for each sample tree and averaged for the two bark samples. Average diameter of axial resin canals was used to estimate the surface area of a single axial resin canals; area = $\pi\ (d/2)^2$ where d be a representative of the average resin canal diameter for each bark sample. Total resin-canal area of each sample tree was calculated by multiplying the total number of axial resin canals and average surface area of single axial resin canals.

2.7. Data analysis

Descriptive statistics was used to describe the bark structure, network and distribution of resin secretory structures in the bark and wood of *B. neglecta*. Pearson̕s correlation analysis was used to determine the relationship between bark thickness, and axial resin canals characteristics with tree DBH. Test of the normal distribution was performed using Shapiro-Wilk. SPSS (PASW 19.0 for Windows statistical software package) was used for the analysis.

3. Results and Discussion

Anatomical description of the bark of *B. neglecta* including the network of resin-secretory structures and distribution of resin canals throughout the inner bark, the relationship between axial resin canals and tree size (DBH) *of B. neglecta* from the Borana zone is presented.

3.1. Bark and resin-secretory structures of Boswellia neglecta

The average bark thickness of *B. neglecta* is 12.83 mm (sd=3.4), ranging from 7.98-22.08 mm at breast height. The equivalent of 20 % of the total tree radius (Table 1). The bark of *B. neglecta* consists of two bark layers, defined as inner and outer bark layer (fig. 1). There were differences in bark thickness between the inner and outer bark layers. The average thickness of the inner bark was 11.05 mm (sd=3.19), it covers about 87.51 % of the total bark and the range was between 6.64 to 20.15 mm (Table 1). Figure 1 shows an overview bark, a closer view on the inner bark is given in fig. 2. The inner bark located adjacent to the vascular cambium composed of living tissues and was characterized by the occurrence of multiseriate tangential bands of sieve tubes, sclerenchyma fibers and axial parenchyma cells in a regular pattern, which are crossed by phloem rays (Fig 1). With increasing distance from the cambium, rays get discontinues and the regular pattern of alternating sieve tubes and sclerenchyma layers is less obvious (fig. 2). Resin canals are imbedded in the parenchyma bands. In most observed trees parenchyma cells get enlarged and form wedges pattern called dilatation (fig. 1). The degree on the presence of dilatation varies between trees but tends to be more obvious in trees with thicker inner bark. Nevertheless, the transition between the part of inner bark which is not affected by dilatation and the part affected by dilatation was not distinct. The outer bark layer consists of a periderm and is hence formed as secondary protective tissue. The periderm is a multi-layered tissue system consisting of a (usually large) phoellem part with regular cells. The outer bark of *B. neglecta* is much thinner than the inner bark, on average, 1.62 mm (sd=0.71) ranging from 0.23-2.93 mm.

The bark of the *B. neglecta* trees showed a scaly outer surface and greenish inner bark. The visual appearance was similar to other Boswellia spp (Lemenih and Teketay. 2003). The inner bark thickness raged between 6.64 and 20.15 mm with an average of 11.05 mm. The thickness of the inner bark was indeed different from *B. papyrifera*. Tolera et al., (2013) reported an average of 17.2 mm inner bark thickness in *B. papyrifera*. It is also lower than the average inner bark thickness of other Boswellia spp growing in the semi-arid areas. The outer bark of *B. neglecta* is much thinner than the inner bark, on average, 1.62 mm. In *B. neglecta*, much larger tree has relatively thicker bark and more bark cross- sectional area. The same feature was shown in *B. papyrifera* (Tolera, 2013).

As expected, axial and radial resin canals found in the inner bark of *B. neglecta*. On the transversal sections, axial resin canals were laid unevenly throughout out the inner bark, lined by a thin layer of epithelial cells (Figure 1). But, a few axial resin canals that are encircled by thick-walled epithelial cells occurred particularly in close to the outer bark , which may show the non-functionality of epithelial cells (Oven and Torelli, 1999). The overall total average number of axial resin canals at cross section area of samples tree was 1540.62 and the overall mean density of axial resin canals was 0.36 per mm^2. These numbers are lower than that of *B. papyrifera* (average total number of axial resin canals was 9887and average density of axial resin canals was 0.86 per mm^2) reported by Tolera (2013b). This difference could be due to the genetic and micro-soil variation(Langenheim, 2003). As resin synthesized and accumulated in specialized secretory structures (Langenheim, 2003), the difference in resin canal number and density between the two Boswellia species may show the difference in abilities to produce and store resin.

This study's result showed that both axial and radial resin canals are present on the tangential sections of inner bark, with the axial resin canals connection by the radial resin canals. This may indicate how resin flows in the bark of Boswellia trees (Tolera et al., 2013). Whereas, in the wood, the result shows that there is not any type of resin secretory structures. In contrast, in other species, for instance in the wood of *Pinus balepensis*, both axial and radial canals were abundantly found, with the axial canals connected by the radial (Werker and Fahn, 1969). Although axial and radial resin canals are interconnected in the bark of *B. neglecta*, it is not clear how the canal network of bark is connected to the wood, but there is a continuity of the ray tissues. In the wood of *P. pinasters*, (Wu and Hu, 1997) study's result, however, indicated that radial and axial resin canals connected by anastomosis. Likewise, in *B. papyrifera* (Tolera et al., 2013) study, result clearly showed that axial and radial resin canals form a three-dimensional network in the nearly cambium (intact zone), and radial resin canals were provided to connect the canal network of bark to wood.

3.2. From resin canals to resin-secretory system

In the wood of *B. neglecta* no resin canals nor axial, nor radial were observed. In the inner bark of B. *neglecta,* axial and radial resin canals (in the rays) are present and can be studied on transversal and tangential sections of the inner bark, respectively. Figure 2 shows that on the transversal sections, axial resin canals were scattered throughout the inner bark, surrounded by a single layer of epithelial cells, and imbedded in multi-seriate sheaths of axial parenchyma. Close to the vascular cambium, most of the axial resin canals are encircled by non-lignified epithelial cells (appearing blue in the thin-section as stained by astra blue). Often resin canals appear arranged in tangential rows, with between two to five canals visible on the cross section, which has a tangential width of about 3mm (Figure 2). Inner bark parts at large distance from the cambium sometimes contains axial canals embedded by lignified epithelial cells indicated by red colour). However, only few lignified resin canals were detected in 11 trees. From the tangential sections it became obvious that axial resin canals are connected with radial resin canals, a phenomenon called anastomosis, and resulting in a three-dimensional network of resin canals. In Figure 3, C illustrates how axial resin canals split up and join neighbouring axial resin canals or radial resin canals. The average lumen diameter of the functional axial resin canals is 0,08mm (sd=0.02) and ranges from 0.05-0.12mm. The average density of functional axial resin canals was calculated to 0.33 (sd=0.11), ranging from 0.15-0.73resin canals mm^{-2} (Table 1).

Table 1: Characteristics of bark and axial resin canals of *B. neglecta* trees from Borana, Ethiopia (N = 21)

Variables	Mean ± SD	Min – Max
DBH in (mm)	120 ± 41.33	61-185
Inner-bark thickness in (mm)	11.26 ± 3.19	6.64-20.15
Outer–bark-thickness (mm)	1.62 ± 0.71	0.23-2.93
Total bark thickness (mm)	12.88 ± 3.40	7.98-22.08
Inner-bark-cross-section- area (mm2)	4079.88 ± 2281.82	1206.56-9876.92
Bark cross-section-area in (mm2)	4728.06 ± 2552.06	1378.67-10955.39
Average density of axial canals number in (mm-2)	0.33 ± 0.11	0.15-0.73
Average diameter of resin canal in (mm)	0.08 ± 0.02	0.05-0.12
Total number of resin canals	1540.62 ± 972.90	463.79-4131.25
Total resin-canal-area in inner bark of tree (mm2)	7.69 ± 5.26	1.32-17.82

SD - Standard deviation, Min - minimum and Max – maximum

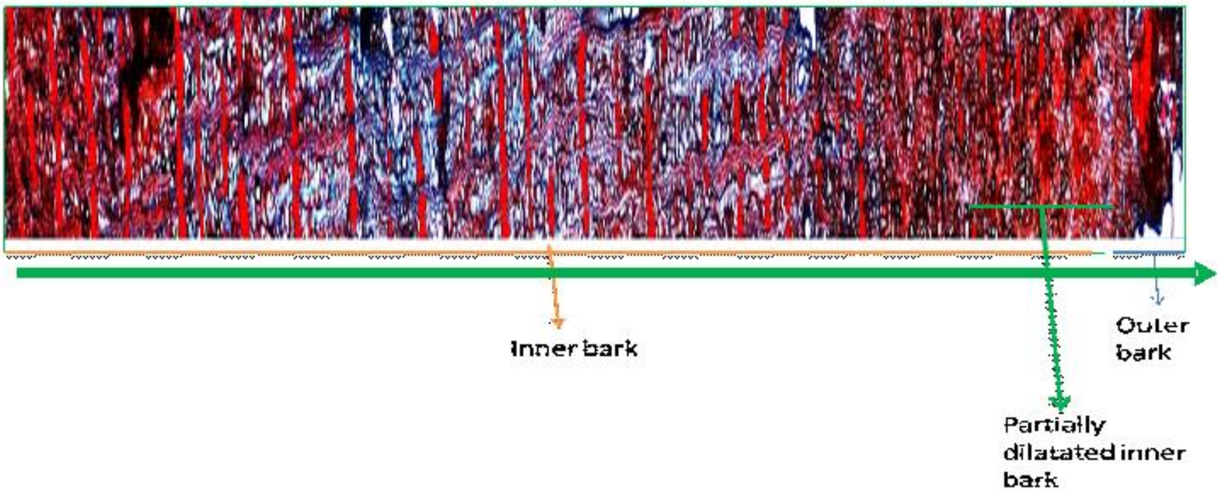

Figure 1: Transversal sections through the bark of B. neglecta. The cambium is located left. The inner bark layer forms the major part of the bark, the outer bark layer is much thinner (see text for wood anatomical description).

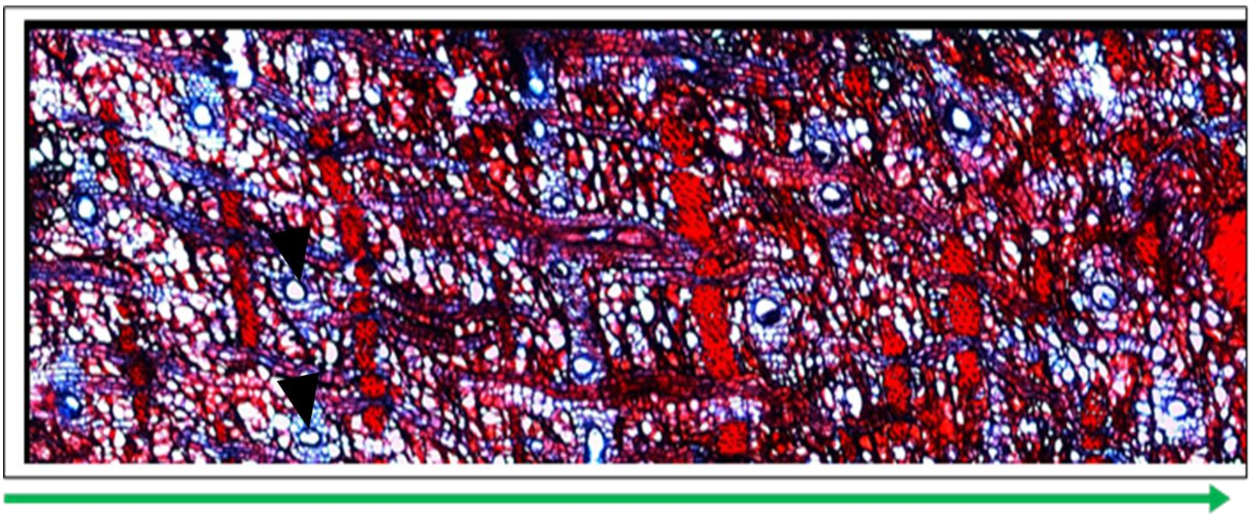

Figure 2: Microscopic view of the inner bark and resin secretory structures of *B. neglecta*: The green arrow points the inner bark structures that arises from nearly cambium towards the outer bark; some resin canals indicated by black arrow heads.

Left, cross section of inner bark with the arrow (A) indicating a resin canal surrounded by non-lignified epithal cells (dark blue) and parenchyma cells; middle, tangential section showing a resin canal in a ray encircled by non-lignified epithelial cells (B); right, tangential section illustrating connection between axial – and possible radial - resin canals called anastomosis (C)

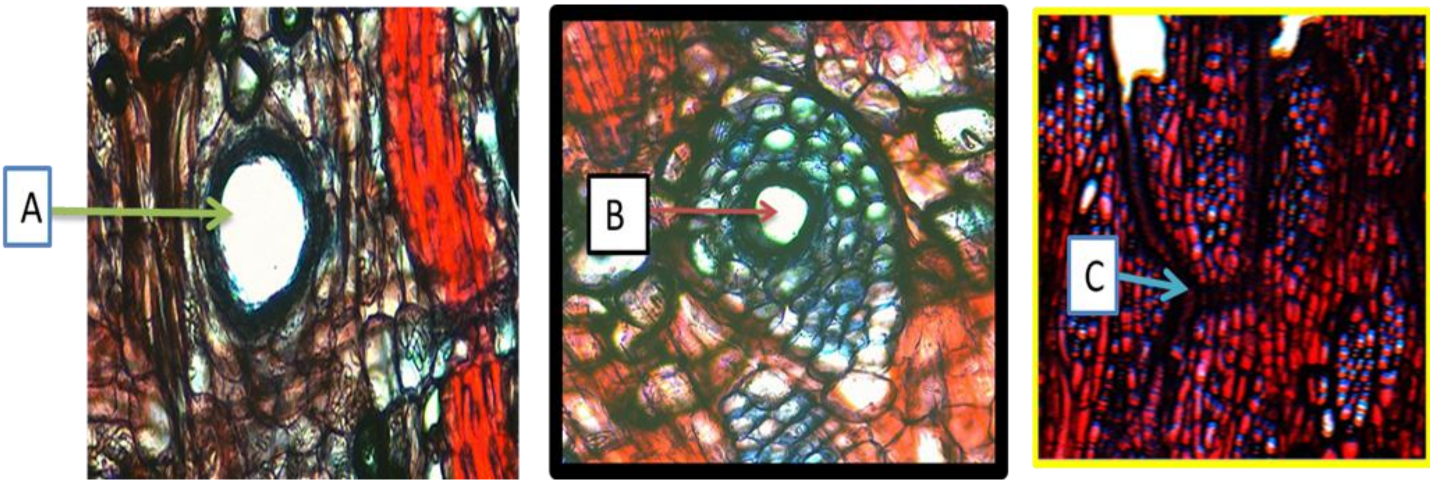

Figure 3: Resin-secretory system in the inner bark of B. neglecta

3.3. Distribution of axial resin canals across the inner bark

In the inner bark mainly functional resin canal were detected. The average density of functional axial resin canals per mm^2 is 0.33 and ranges between 0.15-0.73 per mm^2. The average density of non-functional axial resin canals is considerably lower and amounts to 0.06 per mm^2 and ranges between 0 and 0.12 numbers per mm^2. Functional axial resin canals were unevenly distributed across the inner bark. It has to be mentioned that the general low number of observation makes it difficult to indicate trends. There is however, no obvious and systematic change in resin-canal density across the inner bark. Non-functional axial resin canals only occur with increasing a distance from the cambium.

In this study, it was hypothesized that the distribution of axial resin canals in density exhibit directional changes throughout the inner bark as a result of dilatation, like found in *B. papyrifera*. Tolera et al. (2013) found a gradient in resin-canal density throughout the inner bark with a higher density of axial resin canals close to the vascular cambium and a decrease in resin canal number with increasing distance from the cambium as a result of dilatation. Although dilatation was also observed in *B. neglecta*, it was less intense than in *B. papyrifera*, where the authors could even separate the inner bark into three sections: intact, partly dilatated and strongly dilatated (Tolera et al., (2013). The inner bark of B. *neglecta* exhibit less secondary changes in outer parts, even in thick-bark trees, and the occurrence of dilatation are limited which results in a more even distribution of resin canals, which moreover occur in much lower density in comparison to *B. papyrifera* (Fig.3A). The slight variation in density of axial resin canals in *B. neglecta* can be explained by the limited number of canals, which could be measured in this study. In addition, such uneven distribution may be the result of seasonal variation of resin-canal production (Langenheim, 2003). Internal and external factors such as radial growth rate, wind, pressure, temperature, precipitation, and photoperiod may not only influence the structure of the wood of *B. neglecta* (Mokria, unpubl.Results) but also influence the distributions of resin canals in the bark (Fahn and Zamski, 1970; Wimmer and Grabner, 1997; Zamski, 1972). In *Pinus abies* and *Pinus sylvestris*, density and distribution of axial resin canals were influenced by climatic conditions (Wimmer and Grabner, 1997). More axial resin canals produced under above-normal temperatures and drought stress. Similarly, axial resin canal density of *Pinus taeda* responds to climatic variation (Blanche et al., 1992).

Axial resin canals surrounded by lignified epithel cells have been found in the inner bark with increasing distance from the cambium. The occurrence of these possibly non-functional axial canals in the outer parts of the inner bark may be an effect of physiological age (Rosner and Hannrup, 2004). Another, more likely factor is the effect of dilatation on the epithelial cells (Bannan, 1936; Tolera et al. 2013). Dilatation has been observed in *B. neglecta*, although to a much less extend than in *B. papyrifera*. The degree of dilatation can strongly vary between species. Heavy dilatation has been occurred on the bark of several mainly thick-barked tree species, for instance in *Quercus faginea* Lam (Teresa et al., 2013). The formation of dilatation is due to increasing tangential strain as increasing of tree stem diameter (Evert and Eichhorn, 2006; Quilhó et al., 2013).

3.4. Relationship between bark thickness, and axial resin canals characteristics with tree DBH

Inner bark thickness is significantly related to tree diameter (r=0.68, p<0.001), meaning that inner bark size increases with tree size (Table 2). The average density and diameter of axial resin canals in the inner bark has found to be not significantly related to inner bark thickness respectively, r=-0.076, r=0.107 (p>0.05) (Table 2). However the total area of axial resin canals in bark cross-sectional area have significant positive correlated to DBH, r=0.79, (p<0.001).

Table 2: Pearson correlation coefficient (r) on resin canal and selected tree characteristics of B. neglecta (N=21)

Variables	Inner-bark thickness in(mm)	Axial resin canal density mm^{-2}	Diameter of axial resin canal in (mm)	Total resin canal area in (mm^2)
Inner-bark thickness in(mm)		-0.08	0.11	
DBH (mm)	0.68***			0.79***

* Correlation is significant with P<0.05, **Correlation is significant with P<0.01
*** Correlation is significant with P<0.0001) (table 2).

In this study, the relationships between resin canal (average number, density, diameter and total area of axial resin canal) characteristics in bark cross-section and selected tree (DBH) characteristics was assessed for 21*B. neglecta* sample trees. Inner bark thickness is increased with tree size (DBH). Total area of axial resin canals was highly significant (p<0.001) positively related to the DBH (Fig 4. D).This is to mean that tree with higher stem DBH had a more total area of axial resin canals on the cross sectional bark of *B. neglecta*. The fact that the bark of larger trees contains more resin-producing canals is supported by earlier results (Lorio and Sommers, 1986; Novick et al., 2012) which suggest that larger trees protect themselves by thicker bark with more active resin canals. Likewise, in *Pinus pinaster* (Rodríguez-García et al.(2014a) suggested that trees with better height and diameter growth would allocate their carbon budget to the defence system by increasing secretory structure formation, such as resin canals. Gebrehiwot (2003) also stated that higher resin canals from larger trees may result from larger photosynthetic carbon-acquisition capacities. As resin canal density and size did not significantly decrease through the bark thus means that larger trees are a better to tape.

Although total area of axial resin canals linearly scales with the stem diameter, the average density and diameter of axial resin canals can vary a lot per tree and was not significantly related to bark thickness. For other species, however, average density of axial resin canals was correlated positively with the stem diameter as well as with bark cross-sectional area, for example for *B. papyrifera* (Tolera et al., 2013). For *Pinus taeda* it was reported that density and diameter of resin canals are influenced by the age and radial-growth rate (DeAngelis et al., 1986).

4. CONCLUSION

In this study, bark structure, the characteristics of resin canals and the network of the resin-secretory structures in the *B. neglecta* trees were described. The axial and radial resin canals are present in the inner bark, and are interconnected by radial canals. The average density of axial resin canals is lower, i.e.0.33 mm-2, than has been found in *B. papyrifera*. No resin secretory structures found in the wood of *B. neglecta*. The size and density of resin canals were distributed unevenly across the inner bark. Moreover, inner bark thickness and total resin canal area linearly increases with tree diameter. It can be assumed that larger diameter trees conation much more resin- producing canals than smaller trees with thinner bark.

From earlier Boswellia tree studies, it is evident that frankincense is present in the resin secretory system as performed resin (Tolera et al., 2013). Hence, the present results suggest that selecting and tapping of larger trees might partially contribute to obtain comprehensive frankincense yield (Tadesse et al., 2004). Such information is also important in developing tapping techniques for individual trees (Ella and Tongacan, 1992; Tolera, 2013*).* However, further research is required to introduce new tapping technology for a sustainable frankincense production.

This study was conducted as a follow-up study of the research by Tolera et al. (2013a) on *B. papyrifera*. We used a comparable sampling technique and took two bark samples per tree with a tangential width of 3 mm. As the density of resin canals was considerable lower in *B. neglecta* (average: 0.33 per mm^2) in comparison to *B. papyrifera* (average: 0.86 per mm^2) we could only measure a limited number of resin canals in the inner bark. This made it difficult to define a gradient in resin-canal density across the inner bark and quantify the degree and variation in dilatation between trees. Therefore, more and/or larger bark sample should be collected to verify the results of this study.

Although axial and radial resin canals interconnections are recognized, a more comprehensive study of the degree of interconnection between radial and axial resin canals throughout the inner bark based on consecutive tangential thin section is necessary. This will enable to detect which part of the resin-secretory system in the inner bark is actually functional and where disruptions occur. This could also verify that resin canals with lignified epithal cells are non-functional.

Resin yield, the relationship between resin yield and resin canal characteristics as well as tree characteristics are important traits for selection and formulation of siliviculatural guidelines for tapping *B. neglecta*.

REFERENCE

Angassa, A., and G. Oba, G. (2008). Herder Perceptions on Impacts of Range Enclosures, Crop Farming, Fire Ban and Bush Encroachment on the Rangelands of Borana, Southern Ethiopia. *Human Ecology* 36(2):201-215.

Bannan, M. (1936). Vertical resin ducts in the secondary wood of the Abietineae. *New Phytologis*t 35(1):11-46.

Blanche, C.A., Lorio Jr, P.L., Sommers, R.A., Hodges, J.D and Nebeker T.E. (1992). Seasonal cambial growth and development of loblolly pine: Xylem formation, inner bark chemistry, resin ducts, and resin flow. F*orest Ecology and Management* 49(1–2):151-165.

DeAngelis, J., Nebeker, T. and Hodges, J. (1986). Influence of tree age and growth rate on the radial resin duct system in loblolly pine (*Pinus taeda*). *Canadian Journal of Botany* 64(5):1046-1049.

Ehrlich, J., Wu, X., Lee, I., Hu, Z.-Y., Röckel, H., Marder, S. and Perry. J. (1997). Two-photon absorption and broadband optical limiting with bis-donor stilbenes. Optics Letters 22(24):1843-1845.

Eshete, A., Sterck, F.J. and Bongers, F. (2012). Frankincense production is determined by tree size and tapping frequency and intensity. *Forest ecology and management* 274:136-142.

Eshete, A., Teketay, D. and Hulten, H. (2005). The socio-economic importance and status of populations of *Boswellia papyrifera* (Del.) Hochst. in northern Ethiopia: the case of North Gonder Zone. Forests, trees and livelihoods 15(1):55-74.

Evert, R.F. and Eichhorn. S.E.(2006). Esau's Plant Anatomy: Meristems, Cells, and Tissues of the Plant Body: Their Structure, Function, and Development: Third Edition. 1-601 p.

Fahn, A., and Zamski, E. (1970). The influence of pressure, wind, wounding and growth substances on the rate of resin duct formation in *Pinus halepensis* wood. Israel Journal of Botany 19(2/3):429-446.

FAO (Food and Agriculture Organization). (1995). Gums, resins and latexes of plant origin. Non-Timber Forest Products 6.

Ford, R., Api, A. and Letizia, C. (1992). Monographs on fragrance raw materials. Food and chemical toxicology (ISSN 0278-6915 30.

Gebrehiwot, K. (2003). Ecology and management of *Boswellia papyrifera* (Del.) Hochst. dry forests in Tigray, Northern Ethiopia. Cuvillier.

Gemedo-Dalle, T., Maass, B.L. and Isselstein, J. (2005). Plant biodiversity and ethnobotany of Borana pastoralists in Southern Oromia, Ethiopia. *Economic Botany* 59(1):43-65.

Gizaw, A.W. (2006). Population status and socio-economic importance of gum and resin Bearing species in Borana lowlands, southern Ethiopia, School Of Graduate Studies, Addis Ababa University.

Greene, D. (1993). Gold, frankincense, myrrh, and medicine. *North Carolina medical journal* 54(12):620.

Groom, N. (1981). Frankincense and myrrh: a study of the Arabian incense trade. Longman London.

Khan, I.A., and Abourashed, E.A. (2011). Leung's encyclopedia of common natural ingredients: used in food, drugs and cosmetics. John Wiley & Sons.

Langenheim, J. (1994). Higher plant terpenoids: a phytocentric overview of their ecological roles. J*ournal of chemical ecology* (USA).

Langenheim, J.H. (2003). Plant resins: chemistry, evolution, ecology, and ethnobotany. Timber Press Portland, OR.

Lemenih, M. (2011). Opportunities and challenges for sustainable production and marketing of gums and resins in Ethiopia. CIFOR.

Lemenih, M., and Kassa, H. (2011). Opportunities and challenges for sustainable production and marketing of gums and resins in Ethiopia. Cifor.

Lemenih, M., and Teketay, D. (2003). Frankincense and myrrh resources of Ethiopia: II. Medicinal and industrial uses. SINET: *Ethiopian Journal of Science* 26(2):161-172.

Lemenih, M., Wiersum, K., Woldeamanuel, T., and Bongers, F. (2011). Diversity and dynamics of management of gum and resin resources in Ethiopia: a trade-off between domestication and degradation. Land degradation &development.

Lorio, P.L., and Sommers, R.A. (1986). Evidence of competition for photosynthates between growth processes and oleoresin synthesis in Pinus taeda L. *Tree physiology* 2(1-2-3):301-306.

Mengistu, T., Sterck, F.J. Anten, N.P., and Bongers, F. (2012). Frankincense tapping reduced photosynthetic carbon gain in *Boswellia papyrifera* (Burseraceae) trees. *Forest ecology and management* 278(Complete):1-8.

Nagy, N., Franceschi, V. Solheim, H. Krekling, T., and Christiansen, E. (2000). Wound-induced traumatic resin duct development in stems of Norway spruce (Pinaceae): anatomy and cytochemical traits. *American Journal of Botany* 87(3):302-313.

Novick, K., Katul, G. McCarthy, H., and Oren, R. (2012). Increased resin flow in mature pine trees growing under elevated CO2 and moderate soil fertility. *Tree physiology* 32(6):752-763.

Oven, P., and Torelli, N. (1999). Response of the cambial zone in conifers to wounding. *Phyton-horn-* 39(3):133-138.

Quilhó, T., Sousa, V. Tavares, F., and Pereira, H. (2013). Bark anatomy and cell size variation in *Quercus faginea*. *Turkish Journal of Botany* 37(3):561-570.

Rijkers, T., Ogbazghi, W. Wessel, M., and Bongers, F. (2006b). The effect of tapping for frankincense on sexual reproduction in *Boswellia papyrifera*. *Journal of applied ecology* 43(6):1188-1195.

Rodríguez-García, A., López, R. Martín, J.A. Pinillos, F., and Gil, L. (2014a). Resin yield in *Pinus pinaster* is related to tree dendrometry, stand density and tapping-induced systemic changes in xylem anatomy. 313:47-54.

Rosner, S., and Hannrup, B. (2004). Resin canal traits relevant for constitutive resistance of Norway spruce against bark beetles: environmental and genetic variability. *Forest Ecology and Management* 200(1):77-87.

Schweingruber, F. H., Bornmer, A., Schulze, E., (2006). Atlas of woody plant stems: evolution, structure and environmental modifications. Berlin: Springer-Verla*g*.

Tadesse, W., Desalegn, G. and Alia. R., (2007a). Natural gum and resin bearing species of Ethiopia and their potential applications. Investigación Agraria: Sistemas y Recursos Forestales 16(3):211-221.

Tolera, M., Menger, D., Sass-Klaassen, U., Sterck, F.J., Copini, P., and Bongers, F. (2013). Resin secretory structures of *Boswellia papyrifera* and implications for frankincense yield. *Annals of Botany* 111(1):61-68.

Tolera, M., (2013b). Frankincense yield is related to tree size and resin-canal characteristics. PhD thesis, Wageningen University and Research Center, the Netherlands.

Tucker, A.O. (1986a). Frankincense and myrrh.

Wahab, S.M.A., Aboutabl, E.A., El-Zalabani, S.M., Fouad, H.A., De Pooter, H.L. and El-Fallaha, B. (1987). The essential oil of olibanum. Planta Med 53(4): 382384.

Werker, E., and Fahn, A. (1969). Resin ducts of *Pinus halepensis* mill.–their structure, development and pattern of arrangement. *Botanical Journal of the Linnean Society* 62(4):379-411.

Wimmer, R., and Grabner, M. (1997). Effects of climate on vertical resin duct density and radial growth of Norway spruce [*Picea abies* (L.) Karst.]. Trees 11(5):271-276.

Worku, A. (2006). Population status and socio-economic importance of gum and resin bearing species in Borana Lowlands, southern Ethiopia.

Worku, A., Lemenih, M., Fetene, M., and Teketay, D. (2011). Socio-economic importance of gum and resin resources in the dry woodlands of Borana, southern Ethiopia. Forests, trees and livelihoods 20(2-3):137-155.

Worku, A., Teketay, D., Lemenih, M., and Fetene, M. (2012). Diversity, regeneration status, and population structures of gum and resin producing woody species in Borana, Southern Ethiopia. Forests, trees and livelihoods 21(2):85-96.

Wu, H., and Hu, Z.-h.. (1997). Comparative anatomy of resin ducts of the Pinaceae. Trees 11(3):135-143.

Zamski, E. (1972). Temperature and photoperiodic effects on xylem and vertical resin duct formation in Pinus halepensis Mill. Israel J Bot.

Zhang, C., Fujii, T., Abe, H., Fujiwara, T., Fujita, M., and Takabe, K. (2008). Anatomical features of radial resin canals in Pinus densiflora. *IAWA journal* 29(2):179.